Effect of Early Handling of Turkey Poults on Later Responses to Multiple Dexamethasone-*Escherichia coli* Challenge. 2. Resistance to Air Sacculitis and Turkey Osteomyelitis Complex

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ABSTRACT Dexamethasone (DEX)-induced immunosuppression facilitates *Escherichia coli* pathogenesis leading to lesions of air sacculitis and turkey osteomyelitis complex (TOC). The purpose of this study was to determine if early handling could affect resistance to disease in this model. Seven hundred twenty male turkey poults were handled 0, 1 (1×), or 2 (2×) times daily for the first 10 d after hatch. Handling consisted of gently catching each individual poult, holding it for 10 s, and placing it into a basket. Starting on Day 11 after hatch, half of the birds from each handling treatment were treated with three injections of 2 mg DEX/kg BW on alternating days. On the day of the third DEX treatment, duplicate pens of birds were also inoculated in the air sac with 0 or 50 cfu of *E. coli*. All DEX-treated birds were given a second series of DEX injections at 5 wk of age, and 10 birds per pen were necropsied 3 wk later. Surviving birds were treated with a third series of DEX injections at 10 wk of age. Two weeks later, all surviving turkeys were necropsied. All mortalities and necropsied birds were scored for air sacculitis and examined for TOC lesions. All livers, air sacs, and TOC lesions were cultured for bacteria. There was increased mortality after the first series of DEX treatments of birds handled 2×. After the second series of DEX treatments, birds handled 1× had increased mortality, incidence of air sacculitis, and recovery of *E. coli* from tissues, whereas 2× handled birds were identical to unhandled controls. After the third series of DEX treatments, handling 1× resulted in decreased air sacculitis scores and decreased incidence of mortality, green liver, TOC lesions, and recovery of *E. coli* from tissues. The effects of early handling of turkey poults were variable, depending on the number of DEX treatments and the age of the birds. These results suggest that early handling can affect the susceptibility of stressed turkeys to *E. coli* air sacculitis and TOC and that differences in susceptibility may be influenced by age and individual variability in the stress response.

(*Key words*: turkey, osteomyelitis, stress, handling, *Escherichia coli*)

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INTRODUCTION

Turkey osteomyelitis complex (TOC) is an important cause of field mortality and processing plant condemnations. This disease syndrome has been defined by the Food Safety Inspection Service (FSIS), USDA, to include any normal-appearing processed turkey carcasses that have a green liver and contain lesions including arthritis/synovitis, abscesses in soft tissues, and osteomyelitis of the proximal tibia. Birds on the processing line with green or partially green livers are cut in a standard 10-cut procedure to determine whether the other lesions are present (Cook, 1988). The disease is viewed by the FSIS as a potential food safety concern because the infections often contain *Staphylococcus aureus* and *Escherichia coli* that create the potential for food poisoning.

We have developed an experimental model that allows us to reproduce colisepticemia, air sacculitis, and all of the lesions associated with TOC in a high percentage of turkeys by immunocompromising them with injections of the synthetic glucocorticoid, dexamethasone (DEX), followed by air sac inoculation with 50 to 100 cfu of *E. coli* (Huff et al., 1998). This model has suggested that stress, particularly repeated stressful events, may undermine the immunocompetence of genetically susceptible turkeys, resulting in susceptibility to opportunistic bacterial infections (Huff et al., 1999b). The immunosuppressive effects of stress appear to be greater in male birds than in females. We have found that female turkeys are

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Abbreviation Key: DEX = dexamethasone; FSIS = Food Safety Inspection Service (USDA); HPA = hypothalamic pituitary adrenal axis; TOC = turkey osteomyelitis complex; TPB = tryptose phosphate broth.

more resistant to colibacillosis during DEX-*E. coli* challenge than males (Huff et al., 1999a).

TOC is a disease that primarily affects male turkeys between the ages of 9 and 20 wk of age and is not considered a problem in females (Nairn, 1973; Clark et al., 1991; Mutalib et al., 1996). Sex-related differences in the glucocorticoid response to stress have been documented in mammals (Homo Delarche et al., 1991). It is hypothesized that the modulation mediated by glucocorticoids that prevent cytokines generated by the immune response from reaching damaging levels may be downregulated in females, resulting in increased resistance to bacterial infection as well as an increased susceptibility to autoimmune disease (Munck et al., 1984; Kroemer et al., 1988; Khansari et al., 1990). The involvement of the immune and hypothalamic-pituitary-adrenal (HPA) systems in the stress response has led us to question whether behavioral and psychological responses demonstrated in mammalian stress models are applicable to turkeys. When rat pups are handled daily during the first week of life, the development of the HPA responses to a wide variety of stressors is permanently and robustly altered (Meaney et al., 1993). The number of glucocorticoid receptors in the hypothalamus is increased as a result of neonatal handling and has been demonstrated to produce more efficient and stable hormonal responses to stress. The immune systems of these handled rats are protected from the damaging effects of high levels of catabolic adrenal steroids produced in response to a wide variety of stressors, and these changes persist throughout their lifetimes. Similar manipulations, however, have been associated with increases and decreases in disease resistance (Friedman et al., 1969; Moynihan et al., 1992). The purpose of the present study was to determine whether early handling of turkey poults can permanently affect their ability to resist bacterial infection when immunosuppressed with DEX.

MATERIALS AND METHODS

Seven hundred twenty 1-d-old male poults were wing-banded and divided into 24 randomly assigned floor pens. Untreated control birds and birds challenged with *E. coli* only were housed at a density of 15 poults per pen. Those to be treated with only DEX were housed at 30 poults per pen, and those challenged with DEX and *E. coli* were housed at 60 poults per pen. This variation was allowed to compensate for predicted mortality, and the number of birds per pen was relative to the degree of challenge stress. The number of birds assigned to each handling treatment remained consistent. Duplicate pens of each DEX and *E. coli* challenge group were assigned to each of three handling treatments that consisted of gently handling individual birds 0, 1 (1×), or 2 (2×) times daily for the first 10 d after hatch. The handling procedure was standardized, performed in silence, and consisted of gently catching each individual poult, holding it in both hands for 10 s, and then placing it into a basket with the other poults. When all of the poults had been handled, they were returned to the floor pens sequentially, starting with the first pen handled. Handling was performed by nine individuals, who were randomly assigned to pens according to a pre-arranged schedule. Birds were exposed to 23 h of incandescent lighting per day and were provided access ad libitum to a standard turkey starter diet that met or exceeded the nutrient requirements established by the NRC (1994). Feed consumption was determined weekly.

On the eleventh day after hatch, half of the birds were given three intramuscular injections of approximately 2 mg of DEX[3]/kg BW into a thigh muscle on three alternating days. On the day of the third DEX injection, all birds were inoculated in the left cranial-thoracic air sac with 200 μL of sterile tryptose phosphate broth[3] (TPB) or with 200 μL of TPB containing approximately 50 cfu of a nonmotile, lactose-negative strain of *E. coli* serotype O2. The inoculum was prepared by adding two loopfuls of an overnight culture on blood agar to 100 mL of TPB and incubating for 2.5 h in a 37 C shaking water bath. The culture was held overnight at 4 C while a standard plate count was made. The dilution was made and verified with a second plate count.

Morbidity, mortality, lameness, body weights, and feed consumption were monitored for 2 wk postinoculation. Dead birds were collected twice daily and examined for lesions of air sacculitis/pericarditis and were scored on a scale of 1 to 5. The following key, modified from that described by Piercy and West (1976), was used to score lesions of air sacculitis/pericarditis observed in mortalities and at necropsy: 0, no inflammation; 1, opacity and thickening of the inoculated air sac; 2, mild air sacculitis and mild pericarditis; 3, moderate air sacculitis/pericarditis with spread to liver or adominal cavity (perihepatitis/peritonitis); 4, severe fibrinous air sacculitis and severe pericarditis; 5, severe air sacculitis/pericarditis with spread to liver and/or abdominal cavity. The incidence of TOC was determined by subjecting each carcass to the standard 10-cut procedure used to detect TOC by processing plant inspectors. This procedure includes examining hip, knee, and wing joints; cutting the muscles of the thigh and leg; and cutting across the proximal tibiotarsus to inspect for inflammatory lesions. All TOC lesions, as well as the liver and pericardium from every bird, were swabbed with sterile transport swabs (Bacti-Swab[4]) that were cultured aerobically on Columbia blood agar,[4] MacConkey agar,[4] and mannitol salt agar[4] at 37 C for 18 to 24 h. Swabs were brought to the laboratory and cultured usually within 1 to 2 h from the time of necropsy. Characteristic lactose-negative colonies from MacConkey agar plates were further identified using API biochemical test strips[5] and were compared to the inoculated strain.

Surviving birds were raised until 5 wk of age, at which time all birds previously treated with DEX were given another set of three DEX injections as previously de-

[3]Sigma Chemical Co., St. Louis, MO 63178-9916.
[4]Remel, Lenexa, KS 66215.
[5]Analytab Products, Plainview, NY.

TABLE 1. The effect of handling one time (1×) or two times (2×) daily on incidence of mortality of poults that were treated with three injections of 2 mg of dexamethasone (DEX)/kg BW and were inoculated in the air sac with 50 cfu of *Escherichia coli* at 2 wk of age (DEX 1 treatment)

	No *E. coli*		*E. coli*		Handled MEM[2]
	No DEX	DEX	No DEX	DEX	
	(% Mortality[1])				
Not Handled	0.00 ± 0.00	0.00 ± 0.00	16.00 ± 7.48	15.93 ± 3.46[b]	10.47[b]
Handled 1×	0.00 ± 0.00	1.85 ± 1.85	20.83 ± 8.47	25.74 ± 4.37[a]	15.53[ab]
Handled 2×	0.00 ± 0.00	3.77 ± 2.64	29.63 ± 8.96	34.34 ± 4.80[a]	21.57[a]
MEM					
No *E. coli* 1.30[b]	No DEX 11.49[a]				
E. coli 24.42[a]	DEX 17.16[a]				
Probability values					
Handled	0.0050				
DEX	0.0620				
E. coli	0.0001				
Handled × DEX	0.6966				
Handled × *E. coli*	0.1315				
DEX × *E. coli*	0.8383				
Handled × DEX × *E. coli*	0.9810				

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Values indicate the mean ± SE of birds dying within 3 wk postchallenge.

[2]MEM = Main effect means.

scribed. Mortalities were treated as previously described. Three weeks later, 4 birds per pen were bled, and 10 birds per pen were necropsied in the same manner as mortalities.

Surviving birds were maintained until 10 wk of age, at which time remaining DEX-treated birds were given a third set of DEX injections. Two weeks later, four birds per pen were bled, and all surviving birds were killed and necropsied.

All treatments were carried out in duplicate pens, and data collected from mortalities and from necropsied birds were combined for analyses. All percentage data were subjected to arcsine transformation. Pen means for the effects of DEX, *E. coli*, and handling were analyzed for ANOVA as a 2 × 2 × 3 factorial arrangement using the general linear models and least-squares means procedures of SAS software (SAS Institute, 1988). Significance for main treatment effects and their interactions was considered at $P \leq 0.05$, unless otherwise noted.

RESULTS

DEX 1

Examination of mortalities after the first DEX treatment showed no significant differences related to handling on air sacculitis scores or incidence of TOC, green liver, or recovery of bacteria from tissues. However, poults that had been handled 2×/d had higher ($P \leq 0.05$) mean mortality compared with unhandled birds (Table 1). Birds treated with DEX and challenged with *E. coli* had higher mortality when handled 1× or 2× daily. Dexamethasone treatment did not have a significant effect on mortality at 2 wk of age; however, *E. coli* challenge did increase mortality incidence (Table 1).

DEX 2

After the second series of DEX injections, the mean mortality of birds handled 1×/d was increased. Mortality of birds handled 2×/d and given two DEX treatments, but no *E. coli* challenge, was decreased relative to controls and birds handled 1×/d. Birds handled 1×/d and given DEX treatment and *E. coli* had increased mortality relative to those not handled or handled 2×/d. Mean mortality was increased by DEX treatment but not by *E. coli* challenge. There were significant interactions between handling and DEX and between handling and *E. coli* challenge affecting mortality (Table 2).

Air sacculitis scores were similar to the mean scores of birds handled 1×/d being significantly higher than those handled 0 or 2×/d. Birds that were handled 2×/d and given two DEX treatments, but no *E. coli* challenge, had significantly lower air sacculitis scores compared with birds handled 1×/d ($P = 0.001$) but not significantly lower scores ($P = 0.07$) than controls (Table 3). There were no significant effects of handling on TOC scores. DEX treatment increased TOC incidence, but *E. coli* challenge had no effect (Table 4).

DEX 3

After the third series of DEX injections, handling 1× or 2× daily resulted in significantly lower mean mortality (Table 5) and air sacculitis scores (Table 6). Handling 1× daily resulted in lower mean incidence of TOC (Table 7), green liver (Table 8), and recovery of *E. coli* from tissues (Table 9). Handling 1× or 2× daily lowered the air sacculitis scores of birds treated with DEX but not challenged with *E. coli* (Table 6). Handling 1×/d decreased TOC incidence in birds treated with DEX alone, whereas handling 2× daily increased TOC incidence in birds treated

TABLE 2. The effect of handling one time (1×) or two times (2×) daily on mortality of poults that were challenged with air sac inoculation of 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk and again at 5 wk of age (DEX 2 treatment)

	No *E. coli*		*E. coli*		Handled MEM[2]
	No DEX	DEX	No DEX	DEX	
	(% Mortality[1])				
Not Handled	0.00 ± 0.00	32.69 ± 6.57[a]	4.76 ± 4.76	13.68 ± 3.54[b]	16.49[b]
Handled 1×	0.00 ± 0.00	37.74 ± 6.72[a]	0.00 ± 0.00	41.33 ± 5.72[a]	29.31[a]
Handled 2×	0.00 ± 0.00	17.65 ± 5.39[b]	10.53 ± 7.23	24.62 ± 5.38[b]	16.87[b]
MEM					
No *E. coli* 20.18[a]	No DEX 2.29[b]				
E. coli 21.43[a]	DEX 27.11[a]				
Probability values					
Handled	0.0020				
DEX	0.0001				
E. coli	0.7011				
Handled × DEX	0.0244				
Handled × *E. coli*	0.0296				
DEX × *E. coli*	0.3259				
Handled × DEX × *E. coli*	0.3392				

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Values indicate the mean ± SE of birds dying within 3 wk of the second DEX treatment.

[2]MEM = Main effect means.

with DEX and challenged with *E. coli* (Table 7). Handling 1×/d decreased green liver incidence relative to unhandled controls in birds treated with DEX and challenged with *E. coli* (Table 8) and lowered the incidence of recovery of *E. coli* from tissues relative to birds that were unhandled or handled 2×/d (Table 9).

The means of these parameters were all increased by DEX treatment; however, *E. coli* had no effect. There was an interaction between handling and DEX that affected mortality incidence (Table 5), and there were interactions between handling and *E. coli* challenge that affected air sacculitis score (Table 6) and recovery of *E. coli* from tissues (Table 9).

DISCUSSION

As previously reported, the stimulation provided by brief and gentle handling of turkey poults and retaining them in a basket 1× or 2× daily during the first 10 d after hatch can have significant influence, both positive and negative, on BW and feed conversion, organ weights, serum chemistry values, and white blood cell total and

TABLE 3. The effect of handling one time (1×) or two times (2×) daily on air sacculitis scores of 7-wk-old turkeys that were challenged with air sac inoculation of 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk and again at 5 wk of age (DEX 2 treatment)

	No *E. coli*		*E. coli*		Handled MEM[3]
	No DEX	DEX	No DEX	DEX	
	(Air sacculitis score[1,2])				
Not Handled	0.00 ± 0.00	1.00 ± 0.26[ab]	0.64 ± 0.47	0.94 ± 0.32	0.8095[b]
Handled 1×	0.00 ± 0.00	1.68 ± 0.32[a]	0.00 ± 0.00	1.59 ± 0.27	1.3301[a]
Handled 2×	0.00 ± 0.00	0.37 ± 0.22[b]	0.00 ± 0.00	1.12 ± 0.29	0.5926[b]
MEM					
No *E. coli* 0.82[a]	No DEX 0.12[b]				
E. coli 1.05[a]	DEX 1.18[a]				
Probability values					
Handled	0.0039				
DEX	0.0001				
E. coli	0.3515				
Handled × DEX	0.1533				
Handled × *E. coli*	0.3752				
DEX × *E. coli*	0.9537				
Handled × DEX × *E. coli*	0.4268				

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Each bird was given a score of 0 to 5, based on severity of lesions.

[2]Values indicate the mean ± SE of all mortalities and necropsied birds.

[3]MEM = Main effect means.

TABLE 4. The effect of handling poults one time (1×) or two times (2×) daily on the incidence of turkey osteomyelitis complex (TOC) in 8-wk-old turkeys that were challenged with air sac inoculation of 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk and again at 5 wk of age (DEX 2 treatment)

	No *E. coli*		*E. coli*		Handled MEM[2]
	No DEX	DEX	No DEX	DEX	
	(% TOC[1])				
Not Handled	0.00 ± 0.00	33.33 ± 0.08	9.09 ± 0.09	0.29 ± 0.08	0.25[a]
Handled 1×	0.00 ± 0.00	29.57 ± 0.08	0.00 ± 0.00	0.25 ± 0.06	0.21[a]
Handled 2×	0.00 ± 0.00	20.69 ± 0.08	0.00 ± 0.00	0.14 ± 0.06	0.13[a]
MEM					
No *E. coli* 0.21[a]	No DEX 0.02[b]				
E. coli 0.19[a]	DEX 0.25[a]				
Probability values					
Handled	0.1262				
DEX	0.0001				
E. coli	0.5515				
Handled × DEX	0.7477				
Handled × *E. coli*	0.9448				
DEX × *E. coli*	0.5029				
Handled × DEX × *E. coli*	0.9410				

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Values indicate the mean ± SE of all mortalities and necropsied birds.

[2]MEM = Main effect means.

differential counts in the adult animal (Huff et al., 2001). This study suggests that resistance to stress-related disease is also influenced. The results were not clear cut, as handling appeared to exacerbate the effects of DEX challenge in young poults and ameliorate the effects in older turkeys that had survived two or three DEX challenges.

Neonatal handling of rat pups has been shown to reduce the HPA responses to a number of different stressors, and these effects can last through adulthood (Meaney et al., 1993). Handling is thought to affect this neuroendocrine response to stress by increasing the transcription of glucocorticoid receptor genes in the hypothalamus, which in turn increases sensitivity to glucocorticoid negative feedback regulation of corticotropin-releasing factor and vasopressin (Meaney et al., 1993). There is a bidirectional communication between the immune system and the HPA in mammals and birds (Dunn, 1989; Marsh and Scanes, 1994; Biondi and Zannino, 1997), suggesting that the morphological and physiological changes induced by handling may modulate the deleterious consequences known to affect the immune reponse under conditions of stress. However, previous studies in mice and rats have shown that handling during the neonatal

TABLE 5. The effect of handling poults one time (1×) or two times (2×) daily on the incidence of mortality of 12-wk-old turkeys that were challenged with air sac inoculation of 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk, 5 wk, and 10 wk of age (DEX 3 treatment)

	No *E. coli*		*E. coli*		Handled MEM[2]
	No DEX	DEX	No DEX	DEX	
	(% Mortality[1])				
Not Handled	0.00 ± 0.00	100.0 ± 12.0[a]	0.00 ± 0.00	85.3 ± 4.6[a]	72.0[a]
Handled 1×	11.8 ± 11.8	72.2 ± 10.9[b]	0.00 ± 0.00	47.8 ± 10.6[b]	38.8[c]
Handled 2×	0.00 ± 0.00	81.8 ± 8.4[b]	11.1 ± 11.1	81.5 ± 7.6[a]	56.9[b]
MEM					
No *E. coli* 50.0[b]	No DEX 4.3[b]				
E. coli 62.9[a]	DEX 73.9[a]				
Probability values					
Handled	0.0001				
DEX	0.0001				
E. coli	0.0527				
Handled × DEX	0.0154				
Handled × *E. coli*	0.1870				
DEX × *E. coli*	0.2051				
Handled × DEX × *E. coli*	0.9477				

[a–c]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Values indicate the mean percentage ± SE of birds dying within 3 wk of the third DEX treatment.

[2]MEM = Main effect means.

TABLE 6. The effect of handling poults one time (1×) or two times (2×) daily air sacculitis score of 12-wk-old turkeys that were challenged with air sac inoculation of 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk, 5 wk, and 10 wk of age (DEX 3 treatment)

	No *E. coli*		*E. coli*		Handled MEM[3]
	No DEX	DEX	No DEX	DEX	
	(Air sacculitis score[1,2])				
Not Handled	0.00 ± 0.00	1.82 ± 0.43[a]	0.00 ± 0.00	1.28 ± 0.20	1.11[a]
Handled 1×	0.29 ± 0.29	0.78 ± 0.34[b]	0.00 ± 0.00	0.68 ± 0.25	0.52[b]
Handled 2×	0.00 ± 0.00	0.36 ± 0.15[b]	0.22 ± 0.22	1.38 ± 0.32	0.65[b]
MEM					
No *E. coli* 0.59[a]	No DEX 0.10[b]				
E. coli 0.95[a]	DEX 1.09[a]				
Probability values					
Handled	0.0086				
DEX	0.0001				
E. coli	0.7020				
Handled × DEX	0.1548				
Handled × *E. coli*	0.0178				
DEX × *E. coli*	0.6814				
Handled × DEX × *E. coli*	0.3592				

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Each bird was given a score of 0 to 5, based on severity of lesions.

[2]Values indicate the mean ± SE of all mortalities and necropsied birds.

[3]MEM = Main effect means.

period can depress as well as enhance specific and innate immune functions in the adult animal (Moynihan et al., 1990, 1992). The effects of early handling on infectious disease has been shown to vary with respect to the length of handling and with the species, strain, age, and sex of the animal (Moynihan et al., 1992).

Bhatnagar et al. (1996) have shown that in Long-Evans male rats, neonatal handling confers protection against a decrease of both the plaque-forming cell response and antibody titers to sheep red blood cells, which occur when rats that are chronically and intermittently cold-stressed are exposed to a subsequent acute cold stress. An age effect was observed by Lown and Dutka (1987), who reported that C3H mice that were handled from birth to weaning had no changes in the mitogen responses of spleen cells at Day 21 but had significantly higher responses at 60 d of age.

Studies with chickens have shown positive effects of "adaptation to the handler" on antibody production, serum protein levels, BW gain, and resistance to *Mycoplasma gallisepticum* infection (Gross and Siegel, 1979). This adaptation consisted of gentle touching, stroking, and frequent

TABLE 7. The effect of handling poults one time (1×) or two times (2×) daily on incidence of turkey osteomyelitis complex (TOC) in 12-wk-old turkeys that were challenged with air sac inoculation of 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk, 5 wk, and 10 wk of age (DEX 3 treatment)

	No *E. coli*		*E. coli*		Handled MEM[2]
	No DEX	DEX	No DEX	DEX	
	(% TOC[1])				
Not Handled	00.0 ± 00.0	80.0 ± 9.6[ab]	00.0 ± 00.0	65.6 ± 6.1[b]	55.0[a]
Handled 1×	11.8 ± 8.1	55.6 ± 12.1[b]	11.1 ± 11.1	60.9 ± 10.4[b]	40.3[b]
Handled 2×	00.0 ± 00.0	81.8 ± 8.4[a]	11.1 ± 11.1	85.2 ± 7.0[a]	58.3[a]
MEM					
No *E. coli* 45.0[a]	No DEX 5.8[b]				
E. coli 56.8[a]	DEX 70.6[a]				
Probability values					
Handled	0.0203				
DEX	0.0001				
E. coli	0.8860				
Handled × DEX	0.0742				
Handled × *E. coli*	0.4475				
DEX × *E. coli*	0.6753				
Handled × DEX × *E. coli*	0.7984				

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Values indicate the mean ± SE of all mortalities and necropsied birds.

[2]MEM = Main effect means.

TABLE 8. The effect of handling poults one time (1×) or two times (2×) daily on the incidence of green liver of 8-wk-old turkeys that were challenged with air sac inoculation of 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk, 5 wk, and 10 wk of age (DEX 3 treatment)

	No *E. coli*		*E. coli*		Handled MEM[2]
	No DEX	DEX	No DEX	DEX	
	(% Green liver[1])				
Not Handled	0.00 ± 0.00	42.11 ± 11.64	0.00 ± 0.00	40.98 ± 6.35[a]	33.00[a]
Handled 1×	5.88 ± 5.88	38.89 ± 11.82	0.00 ± 0.00	8.70 ± 6.0[b]	14.92[b]
Handled 2×	0.00 ± 0.00	27.27 ± 9.72	11.11 ± 11.11	29.63 ± 8.96[ab]	20.83[ab]
MEM					
No *E. coli* 22.00[a]	No DEX 28.99[b]				
E. coli 25.90[a]	DEX 32.94[a]				
Probability values					
Handled	0.0139				
DEX	0.0001				
E. coli	0.3682				
Handled × DEX	0.2497				
Handled × *E. coli*	0.1476				
DEX × *E. coli*	0.3482				
Handled × DEX × *E. coli*	0.7294				

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Values indicate the mean ± SE of all mortalities and necropsied birds.

[2]MEM = Main effect means.

speaking for 90 to 120 s/d for the first 4 d. For the remainder of the experiment, a hand was extended into the cage for 1 min while the birds were spoken to.

Early handling has been studied in chickens as a means to modify their fear of humans (Jones and Waddington, 1992, 1993; Jones 1993). These studies suggest that early handling procedures, analogous to those used in classic mammalian studies, reduce experimenter avoidance and tonic immobility reactions. Reduction of a chick's fear of humans is also accomplished by placing a hand into the cage twice daily or by allowing birds visual contact with other birds that are being handled (Jones, 1993). Such handling has also been shown to produce significant changes in brain physiology and morphology, including a decrease in forebrain γ-aminobutric acid ($GABA_A$) receptors and a decrease in $GABA_A$ release from brain tissue but no changes in benzodiazepine receptors (Fluck et al., 1997).

TABLE 9. The effect of handling poults one time (1×) or two times (2×) daily on recovery of *Escherichia coli* from tissues of turkeys that were challenged with air sac inoculation of 50 cfu of *Escherichia coli* at 2 wk of age and were treated with 2 mg dexamethasone (DEX)/kg BW at 2 wk, 5 wk, and 10 wk of age (DEX 3 treatment)

	No *E. coli*		*E. coli*		Handled MEM[3]
	No DEX	DEX	No DEX	DEX	
	(% Recovery of *E. coli*[1,2])				
Not Handled	0.00 ± 0.00	68.42 ± 10.96	10.00 ± 10.00	75.41 ± 5.56[a]	60.00[a]
Handled 1×	5.88 ± 5.88	55.56 ± 12.05	0.00 ± 0.00	39.13 ± 4.35[b]	29.85[b]
Handled 2×	0.00 ± 0.00	54.55 ± 10.87	22.22 ± 14.70	77.78 ± 8.15[a]	48.61[a]
MEM					
No *E. coli* 36.0[a]	No DEX 5.80[b]				
E. coli 56.83[a]	DEX 65.29[a]				
Probability values					
Handled	0.0001				
DEX	0.0001				
E. coli	0.2412				
Handled × DEX	0.1975				
Handled × *E. coli*	0.0475				
DEX × *E. coli*	0.7370				
Handled × DEX × *E. coli*	0.9276				

[a,b]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]Values indicate the mean ± SE of all mortalities and necropsied birds.

[2]Livers, air sacs, and turkey osteomyelitis complex lesions from each bird were cultured on MacConkey agar, and representative lactose negative colonies were further identified using biochemical characteristics. Data are the percentage of birds in which the inoculated strain was isolated from any of these tissues.

[3]MEM = Main effect means.

On the other hand, many reports have associated increases in disease incidence related to neonatal handling procedures. Moynihan et al. (1990) reported that mice handled for 2 min/d had decreased response to immunization with a protein antigen and decreased T-cell reponses to conconavalin A. Handled mice die faster in a stress model that includes inoculation with *E. coli* and total deprivation of food and water (Schlewinski, 1975). When handled mice are inoculated with *E. coli*, but not deprived of feed and water, handling from Days 1 to 18 increases mortality, whereas handling from Days 1 to 10 or from Days 10 to 18 had no effect (Schlewinski, 1976). Laban et al. (1995) reported that adult rats that were handled in infancy have a higher incidence and more severe clinical signs of the disease allergic encephalomyelitis than unhandled controls and that there are sex differences in this response, with the overall effect being more pronounced in males. Handling from Days 1 to 28 has been shown to result in increased antibody production in female rats but lower plaque-forming cell responses in male rats (von Hoersten et al., 1993). Finally, Raymond et al. (1986) have demonstrated that the plaque-forming cell response is decreased by handling of the C57BL/10J strain of mice, but there is no effect when BALB/cJ mice are handled.

It appears that there is a high degree of individual variation in the response to handling, related to sex, strain, and age differences and that these variations may be related to differences in the innate fear response. This study suggests that there is a link between susceptibility to respiratory disease and TOC and the function of the HPA axis of turkeys. We have hypothesized that there is a cumulative effect of the various stressors involved in turkey production that can have a profound effect on the immune system of some male birds, leading to decreased disease resistance. We hope this research will encourage producers to reevaluate production practices with an eye on reducing stress whenever possible, particularly stresses inherent in hatching, vaccination, transportation, and brooding of poults. These results also suggest that early stimulation can have lasting effects on the adult immune response and that further study and optimization may lead to methods for increasing liveability in turkey production. Further work in this area may also lead to methods for the genetic selection of turkeys whose response to the stresses of modern poultry production do not impair health and productivity.

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REFERENCES

Bhatnagar, S., N. Shanks, and M. J. Meaney, 1996. Plaque-forming cell responses and antibody titers following infection of sheep red blood cells in nonstressed, acute, and/or chronically stressed handled and nonhandled animals. Dev. Psychobiol. 29:171–181.

Biondi, M., and L. G. Zannino, 1997. Psychological stress, neuroimmunomodulation, and susceptibility to infectious diseases in animals and man: A review. Psychother. Psychosom. 66:3–26.

Clark, S. R., H. J. Barnes, A. A. Bickford, R. P. Chin, and R. Droual, 1991. Relationship of osteomyelitis and associated soft-tissue lesions with green liver discoloration in tom turkeys. Avian Dis. 35:139–146.

Cook, R. E., 1988. Turkey osteomyelitis-synovitis complex. Inspection Standards and Procedures. Technical Services, Food Safety and Inspection Service. U.S. Department of Agriculture, National Agricultural Library, Washington, DC.

Dunn, A. J., 1989. Psychoneuroimmunology for the psychoneuroendocrinologist: A review of animal studies of nervous system-immune system interactions. Psychoneruoendocrinology 14:251–274.

Fluck, E., S. Hogg, R. B. Jones, R. Bourne, and S. E. File, 1997. Changes in tonic immobility and the GABA-Benzpdiazepine system in response to handling in the chick. Pharmocol. Biochem Behav. 58:269–274.

Friedman, S. B., L. A. Glasgow, and R. Ader, 1969. Psychosocial factors modifying host resistance to experimental infections. Ann. N.Y. Acad. Sci. 164:381–392.

Gross, W. B., and P. B. Siegel, 1979. Adaptation of chickens to their handler, and experimental results. Avian Dis. 23:708–714.

Homo Delarche, F., F. Fitzpatrick, N. Christeff, E. A. Nunez, J. F. Bach, and M. Dardenne, 1991. Sex steroids, glucocorticoids, stress, and autoimmunity. J. Steroid Biochem. Mol. Biol. 40:619–637.

Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath, 1998. The effects of dexamethasone immunosuppression on turkey osteomyelitis complex in an experimental *Escherichia coli* respiratory infection. Poultry Sci. 77:654–661.

Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath, 1999a. Sex differences in the resistance of turkeys to *Escherichia coli* challenge after immunosuppression with dexamethasone. Poultry Sci. 78:38–44.

Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath, 1999b. The effect of a second dexamethasone treatment on turkeys previously challenged in an experimental *Escherichia coli* respiratory model of turkey osteomyelitis complex. Poultry Sci. 78:1116–1125.

Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath, 2001. Effect of early handling of turkey poults on later responses to a dexamethasone—*Escherichia coli* challenge. 1. Production values and physiological response. Poultry Sci. 80:1305–1313.

Jones, R. B., and D. Waddington, 1992. Modification of fear in domestic chicks, *Gallus gallus domesticus*, via regular handling and early environmental enrichment. Anim. Behav. 43:1021–1033.

Jones, R. B., and D. Waddington, 1993. Attenuation of the domestic chick's fear of human beings via regular handling: in search of a sensitive period. Appl. Anim. Behav. Sci. 36:185–195.

Jones, R. B., 1993. Reduction of the domestic chick's fear of human beings by regular handling and related treatments. Anim. Behav. 46:991–998.

Khansari, D. N., A. J. Murgo, and R. E. Faith, 1990. Effects of stress on the immune system. Immunol. Today 11:170–175.

Kroemer, G., H. P. Brezinschek, R. Faessler, K. Schauenstein, and G. Wick, 1988. Physiology and pathology of an immunoendocrine feedback loop. Immunol. Today 9:163–165.

Laban, O., M. Dimitrijevic, S. von Hoersten, B. M. Markovic, and B. D. Jankovic, 1995. Experimental allergic encephalomyelitis in adult DA rats subjected to neonatal handling or gentling. Brain Res. 676:133–140.

Lown, B. A., and M. E. Dutka, 1987. Early handling enhances mitogen responses of splenic cells in adult C3H mice. Brain Behav. Immunol. 1:356–360.

Marsh, J. A., and C. G. Scanes, 1994. Neuroendocrine-immune interactions. Poultry Sci. 73:1049–1061.

Meaney, M. J., S. Bhatnagar, J. Diorio, S. Larocque, D. Francis, D. O'Donnell, N. Shanks, S. Sharma, J. Smythe, and V. Viau, 1993. Molecular basis for the development of individual differences in the hypothalamic-pituitary-adrenal stress response. Cell. Mol. Neurobiol. 13:321–347.

Moynihan, J. A., G. J. Brenner, D. Koota, S. Breneman, N. Conen, and R. Ader, 1990. The effects of handling on antibody production, mitogen responses, spleen cell number, and lymphocyte subpopulation. Life Sci. 46:1937–1944.

Moynihan, J. A., G. J. Brenner, R. Ader, and N. Cohen, 1992. The effects of handling adult mice on immunologically relevant processes. Ann. N.Y. Acad. Sci. 650:262–267.

Munck, A. P., M. Guyre, and N. J. Holbrook, 1984. Physiological functions of glucocorticoids in stress and their relationship to pharmacological actions. Endocrinol. Rev. 5:25–44.

Mutalib, A., B. Miguel, T. Brown, and W. Maslin, 1996. Distribution of arthritis and osteomyelitis in turkeys with green liver discoloration. Avian Dis. 40:661–664.

Nairn, M. E., 1973. Bacterial osteomyelitis and synovitis of the turkey. Avian Dis. 17:504–517.

National Research Council, 1994. Nutrient Requirements of Poultry. National Academy Press, Washington, DC.

Piercy, D. W. T., and B. West, 1976. Experimental *Escherichia coli* infection in broiler chickens: course of the disease induced by inoculation via the air sac route. J. Comp. Path. 86:203–210.

Raymond, L. N., E. Reyes, S. Tokuda, and B. C. Jones, 1986. Differential immune reponse in two handled inbred strains of mice. Physiol. Behav. 37:295–297.

SAS Institute, 1988. SAS/STAT® User's Guide: 1988 Edition. SAS Institute Inc., Cary, NC.

Schlewinski, E., 1975. Studies on the influence of psychological factors on the immune system: the result of infantile stimulation on the disease course in neonatal mice with bacterial infection. Z Psychosom. Med. Psychoanal. 21:390–399.

Schlewinski, E., 1976. Influence of psychological factors on the immune system. Changes in susceptibility to infection after infantile stimulation in relationship to age. Z Psychosom. Med. Psychoanal. 22:370–377.

von Hoersten, S., M. Dimitrijevic, B. M. Markovic, and B. D. Jankovic, 1993. Effect of early handling on behavior and immune response in the rat. Physiol. Behav. 54:931–940.